

October 31, 1951.

Dear Ed:

Thanks for your informative letter. It's too bad about phenol not working after all. Englesberg ran into the same problem of differential survival on synthetic and complete medium in his formaldehyde work, but on chemical grounds the effect is not likely to be the same.

Your idea about preventing dispersion of uv-induced mutant clones sounds very useful, but I wonder if you couldn't design a replica plate scheme that would be less cumbersome than the triple-decker sandwiches. For example, one could plate irradiated cells on complete agar, incubate several hours, replicate to minimal-penicillin agar, incubate overnight, then replicate again to complete agar. This plate is then tested for the presence of mutants by replicas to minimal agar:

1. Complete	2. Minimal-Penicillin	3. Complete	4. Minimal
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This will only work if there is not too much carry over of complete medium from 1 to 2, or penicillin from 2 to 3. Also, a few mutant clones may be lost on transfer from 2 to 3 if there are too few cells to be picked up, but this might not be too serious. The identification of auxotroph colonies (3 - 4) is the one thing I am sure of. If, following your outline, surface colonies could be produced, and the penicillinase and complete supplement introduced under the agar, as in *Neurospora*, replica plating could be used to best advantage for the final step, in place of the delayed enrichment.

Is penicillinase necessary? I wonder if one could not work out conditions under which there is adequate spontaneous inactivation (pH conditions) perhaps assisted by the metabolic or penicillinatic activities of the bacteria. To get really prompt and complete removal of the penicillin concentrations ordinarily used, you must have to use a good deal of (expensive and unstable) penicillinase.

One of the Bact. students here who wants to pick up some techniques may try the replica-modification in a reconstruction experiment, but I doubt whether he will be able to pull it off, and in any event, of course, "it's your baby".

Would you tell Mike that our Lac⁺ Glu-Gal- flopped (probably like Mal-Glu-Ps. *putrefaciens*). We were shocked to hear about Rita, but our condolences could hardly be of any help to Mike.

Sincerely,

J. Lederberg